

restricted differentiation potential were the source of the individual differentiated lineages that arose. Indeed, injection of spheres into nude mice did not form teratomas, which are typically observed after the transfer of ESCs or iPSCs. The SP fraction isolated from the spheres was highly tumorigenic, but if this subpopulation indeed comprised ESC-like cells, why did the tumors only show neuronal differentiation and not cartilage, muscle, bone, etc., as seen in ESC-derived teratomas? Furthermore, attempts to isolate iPSC lines from spheres have failed thus far. Based on these deficiencies, the authors do not claim that the spheres contain iPSCs. It is possible, rather, that the ESC-like cells arising in spheres may not have achieved the full autonomy of truly pluripotent ESCs, and instead remain dependent on a specific microenvironment. Intimate cell-cell contacts that are present within the sphere structures and are lost upon culturing under adherent conditions may provide essential maintenance cues. Thus, the suspension culture of MEFs may induce niches that are required to both induce and maintain the reprogramming of the subset of cells that adopt

characteristics of iPSCs and cancer stem cells.

The findings of Liu et al. are important because they add to the ongoing discussion of the origin and significance of stem cells in tumor development (discussed in [Jordan, 2009](#)). One current view suggests that cancer arises from (epi)genetic transformation of normal stem cells into cancer stem cells that support tumor growth, sustain metastasis, and are responsible for treatment failure. An alternative view is that tumor cells can originate from differentiated cells and that the developing tumor creates niches where differentiated tumor cells become reprogrammed to adopt stem cell characteristics. This second concept is mirrored in the plant kingdom, where the sprouting of roots and shoots relies on the creation of novel stem cell niches in a context of differentiated cells ([Scheres, 2007](#)). The current work of Liu et al. suggests that a similar mechanism may operate during tumor development in animals. Finally, should it be possible to culture iPSC lines from the spheres described by the authors, this method may lead to safer protocols for derivation

of iPSCs to be exploited in regenerative medicine and gene therapy of inherited disease.

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Fishing for a WNT-PGE2 Link: β-Catenin Is Caught in the Stem Cell Net-work

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WNT and PGE2 signaling pathways are each associated with stem cell activity in the hematopoietic system. In a recent issue of *Cell*, [Goessling et al. \(2009\)](#) use the zebrafish model to reveal a conserved PKA-dependent mechanism that connects the two pathways via β-catenin, enhancing stem cell proliferation and tissue regeneration.

Novel insight into cancer biology has come from advances in the stem cell field, with the concept of a cancer stem cell suggesting candidate pathways used to target small populations of tumor cells resistant to standard therapies. But the analogy can work both ways, and signaling mechanisms that modulate tumor cell phenotypes might also be applied to regenera-

tive medicine. This insight is one of two lessons learned from a study investigating how prostaglandins (PGE2) modulate the WNT signaling pathway ([Goessling et al., 2009](#)), published recently in *Cell*.

Familial adenomatous polyposis (FAP) is a condition characterized by hundreds of benign colonic polyps, some of which eventually progress to colon cancer. FAP

is caused by mutations of the APC gene, a tumor suppressor that along with Axin, GSK3β, and CK1 comprise the “destruction complex,” which limits the accumulation of stable, typically proliferative β-catenin. In an early case report, [Waddell and Loughry \(1983\)](#) observed that polyps were largely eradicated in several FAP patients given sulindac, an NSAID shown

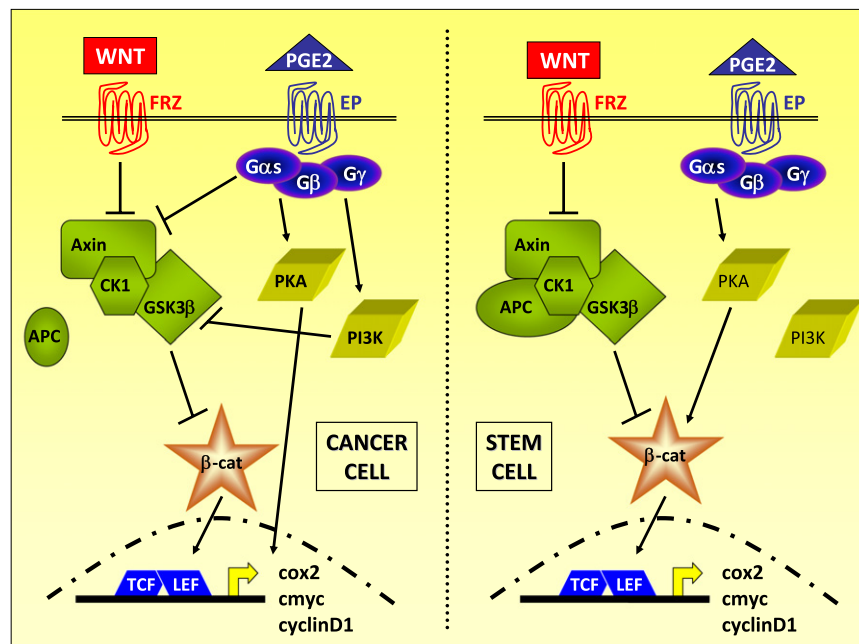


Figure 1. The Same, but Different

In colon carcinoma cell lines (left) and HSCs (right), WNT signaling activates β -catenin through a common canonical pathway, whereby frizzled receptor (FRZ) activation inhibits the destruction-complex-dependent turnover of β -catenin. However, PGE₂ signaling through EP GPCRs may cooperate with WNT via different mechanisms. In a cancer cell, signaling has been shown to affect Axin, PI3K, and PKA activities to indirectly synergize with WNT, while the data from Goessling et al. support a more direct role for PKA targeting of β -catenin. Note that in cancer cell lines, APC is mutant, and this alteration might account for at least some of the observed differences. In both cases, the net result is that effective WNT signaling is dependent on cosignaling from PGE₂ for expression of target genes including Cox2, cMyc, and Cyclin D1, promoting cell survival and proliferation.

in previous animal studies to inhibit chemically induced polyps. NSAIDs are known to inhibit cyclooxygenase (COX) enzymes and thereby limit the synthesis of PGE₂. Ten years later, the efficacy of sulindac for significantly repressing colorectal adenoma in FAP was confirmed by a controlled clinical trial (Giardiello et al., 1993). Cancer researchers have since tried to establish the mechanistic link between APC and NSAIDs by focusing on the two major signaling pathways that are implicated: WNTs and PGE₂. Do they converge and, if so, how?

Cellular levels of β -catenin are controlled by phosphorylation, either directly on β -catenin or indirectly on components of the destruction complex. Suppression of the destruction complex to stabilize β -catenin is well established as the “canonical” pathway downstream of WNT receptor activation. PGE₂ signals instead through GPCRs (EP1-4) to modulate numerous distinct kinase pathways, including those regulated by SRC, PI3K/AKT, and PKA. Cell-culture studies suggest that PGE₂-dependent pathways

can also converge at the level of β -catenin through several indirect mechanisms, including association of the GPCR G α s subunit with Axin, stimulation of the PKA/cAMP pathway, or phosphorylation of GSK3 β (reviewed in Buchanan and DuBois, 2006). Whether these observations in colon cancer cell lines (already mutant for APC) are relevant to stem cell biology had not been addressed. Goessling et al. provide a comprehensive set of data to demonstrate a conserved and widely used stem cell and regeneration pathway in which WNT and PGE₂ collaborate to stabilize β -catenin (Goessling et al., 2009).

The authors focused on the development of long-term repopulating hematopoietic stem cells (HSCs), which arise in vertebrates within the embryonic aorta-gonad-mesonephros (AGM) region and are marked by the expression of the Runx1 transcription factor. In a lead-up study, the Zon group used *runx1* as a marker to screen for small molecules that enhance or suppress HSC generation (North et al., 2007). They identified PGE₂

synthesis as a common pathway associated with multiple chemical hits. Specifically, stabilized derivatives (dmPGE₂) enhance the number of runx1+ cells, while COX2 inhibitors (indomethacin) block the generation of this population. WNT signaling through β -catenin is an established regulator of adult bone marrow hematopoiesis (Malhotra and Kincade, 2009) and the Zon and Moon groups showed recently that it stimulates tissue regeneration (Goessling et al., 2008). Based on the colon carcinoma studies, it made sense to consider whether the two pathways intersect in vivo during HSC development.

First, the investigators used a transgenic zebrafish line carrying a β -catenin-responsive reporter (TOP:dGFP) and found that canonical WNT signals in the AGM and supporting endothelium are enhanced or suppressed by addition of dmPGE₂, or indomethacin, respectively. The number of Runx1+ cells in the AGM was increased following induction of Wnt8 expression, and this effect was blocked by treatment with indomethacin. Therefore, WNT signals enhance generation of HSC, and this outcome is dependent on signals from PGE₂ that promote cell survival and cell proliferation. In a series of tests for epistasis combining specific pathway inhibitors with inducible transgenic lines, the effect of PGE₂ on WNT activity was shown to converge at a common point: the levels of phosphostabilized β -catenin. The ability of PGE₂ to modulate WNT was shown to be dependent on cAMP/PKA signals, and apparently not on PI3K signaling, which may distinguish the stem cell mechanism from at least some cancer cell models (Figure 1). Goessling et al. next show that the pathways cooperate not only during embryogenesis, because synergy is conserved during hematopoietic progenitor cell recovery in irradiated adult fish. The mechanism is also conserved across species, since WNT and PGE₂ combine to promote generation of murine ESC-derived hematopoietic progenitors in vitro and to enhance survival and repopulating activity of bone marrow-derived progenitors in transplanted hosts, even when donor HSCs are exposed to pathway modulators ex vivo. Finally, WNT signaling was demonstrated to also be dependent on PGE₂ activity in the context of zebrafish liver and fin regeneration.

There remain some mysteries to be solved. First, β -catenin is not required for hematopoiesis. Whether this independence is also true for tissue regeneration is not known, although WNT signaling activity may be a more pertinent criterion, since even the β -catenin/ γ -catenin double knockout mice retain WNT-reporter activity through an unknown compensatory mechanism (Jeannot et al., 2008). Second, sustained (chronic) stimulation of either PGE2 or WNT signaling could potentially have the opposite effect and deplete stem cell activity (Kirstetter et al., 2006; Scheller et al., 2006). Therefore, it might be appropriate in a clinical setting to modulate β -catenin through these pathways, but there can be too much of a "good thing." It will be a tricky balancing act to promote regeneration without depletion of stem cell pools or promotion of oncogenesis.

A second lesson provided by this study is that elegant experimental approaches in the zebrafish model system can be combined in imaginative ways to reveal mechanistic insight. The list of techniques includes the use of reporter fish to quantify in vivo signaling activities and stem

cell populations, a repertoire of transgenics for manipulating signaling conditionally, and the use of chemical biology to identify new probes for blocking or enhancing pathways to establish mechanism. While each of the research tools employed here was already available, their combined use applied across multiple organ systems in the embryo and the adult demonstrates a very impressive molecular toolbox for the zebrafish model. The relative ease of applying chemical probes and inducible stimuli (in this case, heat, but many others are conceivable), combined with lineage reporters and tissue regeneration assays, confirm the advantage of testing complicated models in fish before moving on to confirm they are conserved in mammalian systems. The success of Goessling et al. in this sense bodes well for future fishing experiments and the discovery of novel modulators to impact both cancer and stem cell biology.

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One Flew over the Progenitor's Nest: Migratory Cells Find a Home in Osteoarthritic Cartilage

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Articular cartilage is the target tissue of osteoarthritis (OA), a degenerative disease with no cure. In this issue of *Cell Stem Cell*, Miosge and colleagues (Koelling et al., 2009) report that migratory progenitor cells occupy degenerating OA tissue but that this population is not present in healthy cartilage.

In 1740, James Douglas, physician to the Queen of England, was involved in a monumental study on the nature of bones and employed the noted anatomist William Hunter to be his dissector. Douglas died 2 years later, but Hunter persisted, and in 1743 the results of his

observations on the structure of articular cartilage and its diseases were published by the Royal Society (Hunter, 1743). Using a glass lens, Hunter described the unbroken, glossy smoothness of the articular surface, that "it yields to the touch, but restores itself to its former equality of

surface when the pressure is taken off." He also noted, "From Hippocrates to the present age it is universally allowed that ulcerated cartilage is a troublesome thing and that, once destroyed, is not repaired." Until recently, it was considered that, in part, cartilage's poor reparative